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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/680,286	10/06/2003	Marguerite A. Cervin	CL2180USNA	5853

23906 7590 04/10/2007
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EXAMINER

CHOWDHURY, IQBAL HOSSAIN

ART UNIT	PAPER NUMBER
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1652

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	04/10/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary

Application No.

10/680,286

Applicant(s)

CERVIN ET AL.

Examiner

Iqbal H. Chowdhury, Ph.D.

Art Unit

1652

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 14 December 2006.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-8 is/are pending in the application.
- 4a) Of the above claim(s) 4-7 is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1-3 and 8 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____ |
| 3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date <u>02/05</u> . | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

Application Status

Claims 1-8 are currently pending in the instant Office action.

In response to a previous Office action, a non-final requirement (mailed on July 14, 2006), Applicants filed a response and an amendment on December 14, 2006, amending claims 3 and 8 is acknowledged. Claims 4-7 remain withdrawn. Claims 1-3 and 8 are under consideration.

Applicants' arguments filed on December 14, 2006, have been fully considered but are not deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Maintained Claim Rejections - 35 U.S.C. § 112(2nd)

Previous rejection of claim 8 under 35 USC § 112, second paragraph, as being indefinite is maintained. Claim 8 was rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 8 was indefinite and unclear with the recitation "a 1.6 long GI promoter" or "1.5 long GI promoter".

Applicants argue that the terms "a 1.6 long GI promoter" and "a 1.5 long GI promoter" are described in the specification at page 32, line 31 - page 33, line 24 and in SEQ ID NOs: 65-68. Applicants also argue that by referencing SEQ ID NOs: 65-68, the exact sequences of these promoters are available to one skilled in the art in possession of Applicants' specification and the terms, used as descriptors of these promoters, are definite,

Art Unit: 1652

This is not found persuasive because amended Claim 8 still reads on “1.6 long GI promoter” or “1.5 long GI promoter”, and the specification at page 33, line 23 recites “p1.6 long GI (dhaB1_dhaB2_dhaB3_dhaX)”, and “p1.6 long GI (orfY_orfX)”, which are the part of SEQ ID NO: 68 and specification at page 34 line 9 and 18, recites “p1.6 long GI (orfY_orfX_orfW)”, and at page 33 line 13 to 14 recites “p1.5 long GI (dhaB1_dhaB2_dhaB3_dhaX)” and “p1.5 long GI (orfY_orfX_orfW)”, those are more confusing, which does not define clearly what does 1.6 GI promoter or 1.5 GI promoter mean? These phrase used in the claims should be define clearly in the specification as if a skill artisan would practice the claimed invention commensurate to the scope of the claim. If applicant’ are referring "a 1.6 long GI promoter" and "a 1.5 long GI promoter", a specific portion of SEQ IDs: 66 and 68, it is suggested that they refer to them directly. The Examiner assumes that 1.6 long or 1.5 long is 1.6 KB long promoter or 1.5 KB long promoter. However, it is not clear to the Examiner what does GI mean? These phrases are still confusing and unclear to the Examiner. Therefore, the rejection is maintained.

Maintained - Claim Rejections - 35 U.S.C. § 112

Claims 1-3 and 8 are directed to an E. coli strain comprising highly variable genus of genes including: a) disrupted endogenous phosphoenolpyruvate-glucose phosphotransferase system preventing expression of active PEP-glucose phosphotransferase system proteins; b) an up regulated endogenous galP gene encoding active galactose-proton symporter or galactose permease; c) an up regulated endogenous glk gene encoding active glucokinase; and d) a down regulated endogenous gapA gene encoding active glyceraldehyde 3-phosphate dehydrogenase. Claim 2 also directed to said E. coli strain, wherein the disrupted endogenous

Art Unit: 1652

phosphoenolpyruvate-glucose phosphotransferase system comprises one or more of: i) disrupted endogenous *ptsH* gene preventing expression of active phosphocarrier protein; ii) disrupted endogenous *ptsI* gene preventing expression of active phosphoenolpyruvate-protein phosphotransferase; and iii) disrupted endogenous *crr* gene preventing expression of active glucose-specific IIA component and Claim 3 directed to said *E. coli* strain further comprising a disrupted endogenous *arcA* gene preventing expression of active aerobic respiration control protein. In addition to the limitations of claims 1 and 3, claim 8 recites an *E. coli* strain comprising one plasmid comprising a first operon comprising genes encoding glycerol-3-phosphate dehydrogenase and glycerol-3-phosphatase, second operon further comprising 1.6 long GI promoter controlling genes encoding dehydratase and a first subunit of dehydratase reactivation factor, having sequence of SEQ ID NO: 68 that comprises *orfW*.

Applicants argue that an *E. coli* strain having the claimed disruptions, up-regulations, and down-regulations to endogenous genes is well-described in the working examples and throughout the specification, each *E. coli* gene is well-described in the specification (see page 12, line 21 -page 20, line 15), and applicants have provided several biological deposits (see page 11, lines 8-36), detailed working examples are provided, and multiple methods of achieving disruption (distinct from down regulating), up regulation, and down regulation, which are applicable regardless of gene target, are similarly well-described (see, e.g., page 35, line 12 - page 36, line 26). Applicants' also argue that the specification provides a detailed description of the disrupted, up-regulated, and down-regulated endogenous *E. coli* genes, has pointed the skilled person to a number of specific sequences with structural features for each gene, and has fully described methods of gene manipulation useful in practicing the present invention.

Applicants submit that their specification has put the skilled person on notice that Applicants were in possession of the claimed invention at the time of filing.

Applicant's arguments and amendments to claims have been fully considered but are not deemed to be persuasive to overcome the rejection on Written description issues.

Examiner acknowledges the amendment of claim 3 and arguments regarding disruptions, up-regulation and down-regulation of said endogenous genes in said microorganism, however the amendment and arguments do not give enough structural information regarding method of up-regulation or down-regulation of endogenous said genes in said microorganism. The Examiner acknowledges that in pages 35-36, the specification provides some information about up and down-regulation of said genes such as additional copies of said genes or changing promoters or mutating promoters for up-regulation, and deletion, insertion or alteration of coding regions and /or regulatory region including random mutation for down-regulation. However, claims read on any kind of methods for up-regulation, which may include- chemical induction or inactivation of repressor molecules resulting in up-regulation, and for down-regulation, which may include chemical repression or using Si RNA against said genes in said microorganism. Given this lack of description of representative species encompassed by the genus of DNAs used in the methods of making a microorganism having desired disruption, up- or down-regulation function, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

As discussed in the written description guidelines the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of

Art Unit: 1652

species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. A representative number of species means that the species, which are adequately described are representative of the entire genus. **Thus, when there is substantial variation within the genus, one must describe a sufficient structure and variety of species to reflect the representative structure variation within the genus.** Satisfactory disclosure of a representative structure and number depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of species disclosed. For inventions in an unpredictable art, adequate written description of a genus, cannot be achieved by disclosing the structure of small portion of only one species within the genus. The method of disruption, up- or down-regulation of the expression of said genes used to make an E. coli strains having corresponding activity is structurally diverse as it broadly encompasses many different methods having different DNA structures. As such, the disclosure solely of functional features that may or may not present in all members of the genus is insufficient to be representative of the attributes and features of the entire genus. Therefore, the rejection is maintained.

Maintained - Claim Rejections - 35 U.S.C. § 112

Previous rejection of Claims 1-3 and 8 under 35 U.S.C. 112, first paragraph, enablement requirement, is maintained. This rejection has been described in length in previous Office

Art Unit: 1652

Action. Applicant's arguments have been fully considered but are not deemed persuasive for the following reasons.

Applicants argue that the touchstone of the enablement requirement (Wands Factor) is whether the skilled person can make and use the invention without undue experimentation, and applicants submit that the skilled person, in possession of the present application describing *E. coli* strains having the claimed gene disruptions, up-regulations, and down-regulations, in conjunction with well-known protocols of molecular biology, which would have no difficulty in practicing the invention without undue experimentation. Applicants further argue that one skilled in the art would expect some testing, screening, and trial and error to implement the present invention outside the working examples, however, the information presented in the instant application is sufficient to enable one skilled in the art to implement the gene disruptions, up-regulations, and down-regulations needed to practice the invention. Applicants further argue that in the background of the invention biological production of 1,3-propanediol via fermentation of glycerol is well-known and *E. coli* containing the genes responsible for conversion of glycerol to 1,3-propanediol in *Klebsiella pneumonia* and *Citrobacter freundii* are also known. Applicants furthermore, argue that biological methods of 1,3-propanediol production, in addition to well-known chemical production methods, are unsuitable for industrial scale 1,3-propanediol production because they are energy intensive; require the use of an expensive starting material, glycerol; and have low yields. Applicants also argue that applicants have successfully developed an *E. coli* that utilizes a low cost carbon substrate (e.g., glucose) to produce 1,3-propanediol at high yields and the methods for gene disruption, up-regulation, and down-regulation needed to practice Applicants' invention are well-known in the art. Applicants furthermore, argue that this

Art Unit: 1652

invention is related to the biotechnical arts in an extremely well-known organism having disruptions, up-regulations, down-regulations of well-known genes, and the skill level of the artisan is very high and the skilled artisan is therefore very familiar with E. coli strains and well versed in many methods and techniques of gene manipulation and the biotechnical art is an unpredictable art and applicants have depended on the skill and experience of the artisan to implement the invention using gene disruption, up-regulation, and down-regulation methods of their choosing, and it is expected that the artisan would be aware of successful methods of E. coli gene mutations and therefore be capable of implementing the described genetic manipulations in E. coli. Applicants also argue that the breadth of the claim is reasonable given the vast improvement and the ability of skilled artisans to implement the invention in E. coli, it would be unfair to the Applicants to limit their invention to the working examples as the Applicants' specification has provided enough description to allow others in the art to use the present invention with any E. coli having the claimed disruptions, up-regulations, and down-regulations.

Applicant's arguments have been fully considered but are not deemed persuasive to overcome the rejection of claims 1-3 and 8 on enablement issue. The examiner acknowledges the amendment to the claims but disagrees with the applicant's contention that the scope of the claimed invention is adequately described.

Examiner is also acknowledged the applicants analysis of the Wands factors to provide the evidence that the claimed invention is fully enabled. However, claims still read on any E. coli strain comprising a) any disrupted endogenous phosphoenolpyruvate-glucose phosphotransferase system (operon); and b) up regulation of any endogenous galP gene; c) up regulation of any endogenous glk gene; and d) a down regulation of any endogenous gapA gene and disruption of

Art Unit: 1652

any endogenous arcA gene and further comprising any glycerol-3-phosphate dehydrogenase gene and any glycerol-3-phosphatase gene for the production of 1,3-propanediol from glucose.

Claims also still read on any methods of disruption of genes or any methods for up- or down-regulation of said genes in said E. coli microorganism. Examiner acknowledges that in pages 35-36 and in Working Examples, the specification provides some guidance about up- and down-regulation of said genes such as additional copies of said genes or changing promoters or mutating promoters for up-regulation, and deletion, insertion or alteration of coding regions and /or regulatory region including random mutation for down-regulation. However, claims read on any kind of methods for up-regulation, which may include- chemical induction or inactivation of repressor molecules resulting in up-regulation, and for down-regulation, which may include chemical repression or using Si RNA against said genes in said microorganism.

The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of E. coli strains encompassed as the claims do not specify how the endogenous E. coli Pts, galP, gapA, arcA, G3PDH and glycerol 3-phosphatase genes are modified to produce the up/down regulated genes recited.

The scope of the claims is also not commensurate with the enablement provided by the disclosure with regard to the methods of modification (disruption) of E. coli endogenous PEP-PTS genes (ptsH gene, ptsI gene, and crr gene) or arcA gene for modified expression or reduced expression of gapA gene or increased expression of galP and glK gene broadly encompassed by the claims. Since the expression of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence or in the promoter sequence and obtain the desired activity requires a knowledge of and guidance with

Art Unit: 1652

regard to which amino acids in the protein's sequence or the promoter sequence and techniques if any, are tolerant of modification, and detailed knowledge of the ways in which the proteins' expression relates to its function. However, in this case the disclosure is limited to the modification of PEP-PTS system and *arcA* gene by P1 phage transduction (disruption), up regulation of *glk* and *galP* genes by putting strong *Trc* promoter and down regulating endogenous *gapA* gene by replacing ATG start codon with GTG or TTG codon.

The specification also does not support the broad scope of the claims, which encompass methods of using an *E. coli* comprising any disrupted PEP-PTS genes or *arcA* gene, down regulating of any *gapA* gene or up regulating of any *galP* gene or *glk* gene because the specification does not establish: (A) regions of the protein structure which may be modified such that PEP-PTS system proteins and *arcA* gene encoding protein lacks activity and reduced activity of *gapA* gene encoding protein and up regulating activity of *galP* or *glk* gene encoding proteins; (B) the general tolerance of *gapA* or *galP* or *glk* proteins to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any PEP-PTS system proteins, *arcA* protein, *gapA* protein, *galP* or *glk* protein residues with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of any *E. coli* strain having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir, 1988). Therefore, the

rejection is maintained.

Withdrawn-Claim Rejections - 35 USC § 103

Previous rejection of Claims 1-3 and 8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Baez et al. (Determination of 3-deoxy-D-arabino-heptulosonate 7-phosphate productivity and yield from glucose in Escherichia coli devoid of the glucose phosphotransferase transport system, Biotechnol Bioeng. 2001 Jun 20; 73(6): 530-5), Seta et al. (Characterization of Escherichia coli strains with gapA and gapB genes deleted, J. Bacteriol. 1997 Aug; 179(16): 5218-21), Iuchi et al. (arcA (dye), a global regulatory gene in Escherichia coli mediating repression of enzymes in aerobic pathways, Proc Natl Acad Sci U S A. 1988 Mar; 85(6): 1888-92) in view of Emptage et al. (WO 01/12833 A2, publication 2/22/2001, Process for the biological production of 1,3-propanediol with high titer, see IDS) and Payne et al. (US PGPUB 20050147968 A1, publication 7/7/2005, claim priority of 60/374931 of 4/22/2002) is withdrawn in view of applicants amendments of claims and persuasive arguments because Baez et al. do not teach an E. coli strain having reduced expression of gapA and Seta et al. do not teach an E. coli strain with alteration of PEP, galP or glk and both of the reference provide motivation for producing 1,3-propinodiol by using modified E. coli strain. Therefore, the rejection is withdrawn.

Conclusion

Claims 1-8 are pending.

Claims 4-7 are withdrawn.

Claims 1-3 and 8 are rejected.

Art Unit: 1652

Applicants must respond to the objections/rejections in each of the sections in this Office action to be fully responsive in prosecution. Accordingly, **THIS ACTION IS MADE FINAL**. See M.P.E.P. 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 C.F.R. 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 C.F.R. 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Iqbal Chowdhury, Ph.D. whose telephone number is 571-272-8137. The examiner can normally be reached on 9:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 703-272-0928. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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